```
=> d his

     (FILE 'HOME' ENTERED AT 16:05:44 ON 20 JUN 2003)

     INDEX 'ADISCTI, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, AQUASCI,
     BIOBUSINESS, BIOCOMMERCE, BIOSIS, BIOTECHABS, BIOTECHDS, BIOTECHNO, CABA,
     CANCERLIT, CAPLUS, CEABA-VTB, CEN, CIN, CONFSCI, CROPB, CROPU, DDFB,
     DDFU, DGENE, DRUGB, DRUGLAUNCH, DRUGMONOG2, ...' ENTERED AT 16:05:57 ON
     20 JUN 2003
               SEA IDURONIDASE
               ---------
                4   FILE ADISINSIGHT
                7   FILE ADISNEWS
                5   FILE AGRICOLA
                4   FILE ANABSTR
                1   FILE AQUASCI
                5   FILE BIOBUSINESS
               15   FILE BIOCOMMERCE
              411   FILE BIOSIS
               32   FILE BIOTECHABS
               32   FILE BIOTECHDS
              149   FILE BIOTECHNO
               22   FILE CABA
               38   FILE CANCERLIT
              279   FILE CAPLUS
                4   FILE CEABA-VTB
               28   FILE CIN
               11   FILE CONFSCI
               13   FILE DDFB
               18   FILE DDFU
               67   FILE DGENE
               13   FILE DRUGB
               26   FILE DRUGNL
               20   FILE DRUGU
                7   FILE DRUGUPDATES
                1   FILE EMBAL
              354   FILE EMBASE
               78   FILE ESBIOBASE
               10   FILE FEDRIP
                1   FILE FSTA
              235   FILE GENBANK
               29   FILE IFIPAT
                8   FILE JICST-EPLUS
               70   FILE LIFESCI
              355   FILE MEDLINE
              139   FILE PASCAL
                4   FILE PHAR
               21   FILE PHARMAML
               36   FILE PHIN
              107   FILE PROMT
              303   FILE SCISEARCH
               58   FILE TOXCENTER
              152   FILE USPATFULL
                4   FILE USPAT2
                1   FILE VETU
               26   FILE WPIDS
               26   FILE WPINDEX
L1               QUE IDURONIDASE
                 ---------

     FILE 'BIOSIS, MEDLINE, EMBASE, SCISEARCH, CAPLUS, BIOTECHNO, PASCAL,
     PROMT' ENTERED AT 16:07:00 ON 20 JUN 2003
L2            83 S L1 AND (CHINESE HAMSTER OVARY OR CHO OR 2.131)
```

L3 30 DUP REM L2 (53 DUPLICATES REMOVED)

=> d l3 ibib ab 25-30

L3 ANSWER 25 OF 30 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 10
ACCESSION NUMBER: 1992:25281 BIOSIS
DOCUMENT NUMBER: BA93:14556
TITLE: HUMAN ALPHA-L **IDURONIDASE** CDNA ISOLATION AND EXPRESSION.
AUTHOR(S): SCOTT H S; ANSON D S; ORSBORN A M; NELSON P V; CLEMENTS P R; MORRIS C P; HOPWOOD J J
CORPORATE SOURCE: LYSOSOMAL DIS. RES. UNIT, DEP. CHEM. PATHOL., ADELAIDE CHILDREN'S HOSP., NORTH ADELAIDE S.A. 5006, AUSTRALIA.
SOURCE: PROC NATL ACAD SCI U S A, (1991) 88 (21), 9695-9699. CODEN: PNASA6. ISSN: 0027-8424.
FILE SEGMENT: BA; OLD
LANGUAGE: English
AB .alpha.-L-**Iduronidase** (IDUA; EC 3.2.1.76) is a lysosomal hydrolase in the metabolic pathway responsible for the degradation of the glycosaminoglycans heparan sulfate and dermatan sulfate. A deficiency of IDUA in humans leads to the accumulation of these glycosaminoglycans and results in the lysosomal storage disorder mucopolysaccharidosis type I. We have isolated and sequenced cDNA clones containing part of the human IDUA coding region and used PCR from reverse-transcribed RNA to obtain the full IDUA sequence. Analysis of the predicted 653-amino acid precursor protein shows that IDUA has a 26-amino acid signal peptide that is cleaved immediately prior to the amino terminus of the 74-kDa polypeptide present in human liver IDUA. The protein sequence contains six potential N-glycosylation sites. Northern blot analysis with IDUA cDNA detected only a single 2.3-kilobase mRNA species in human placental RNA; however, PCR analysis of fibroblast, liver, kidney, and placental RNA showed the existence of alternatively spliced mRNA from the IDUA gene. Southern blot analysis failed to detect major deletions or gene rearrangements in any of the 40 mucopolysaccharidosis type I patients studied. Expression of a full-length IDUA cDNA construct in **Chinese hamster ovary** cells produced human IDUA protein at a level 13-fold higher than, and with a specific activity comparable to, IDUA present in normal human fibroblasts.

L3 ANSWER 26 OF 30 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 11
ACCESSION NUMBER: 1987:146062 BIOSIS
DOCUMENT NUMBER: BA83:75112
TITLE: PRELIMINARY CHARACTERIZATION OF A **CHINESE HAMSTER OVARY** CELL GLYCOSYLATION MUTANT ISOLATED BY SCREENING FOR LOW INTRACELLULAR LYSOSOMAL ENZYME ACTIVITY.
AUTHOR(S): HALL C W; ROBBINS A R; KRAG S S
CORPORATE SOURCE: DEP. BIOCHEM., JOHN HOPKINS UNIV., SCH. HYGIENE PUBLIC HEALTH, BALTIMORE, MARYLAND 21205, USA.
SOURCE: MOL CELL BIOCHEM, (1986 (RECD 1987)) 72 (1-2), 35-46. CODEN: MCBIB8. ISSN: 0300-8177.
FILE SEGMENT: BA; OLD
LANGUAGE: English
AB A novel screening procedure was developed for isolating **Chinese hamster ovary** cell mutants altered in the early steps of the biosynthesis of asparagine-linked glycoproteins. This procedure identifies cells with low intracellular levels of two lysosomal hydrolases, beta-glucuronidase and alpha-**iduronidase**. One mutant cell line isolated in this way, CHB 11-1-3, has low intracellular levels of seven lysosomal enzymes as compared to wild-type cells. Although CHB 11-1-3 synthesizes mannosylphosphoryldolichol and [Man]5[NAcGlcNH2]2-P-P-lipid, it fails to utilize these lipid intermediates to make normal amounts of [Glc]3[Man]9[NAcGlcNH2]2-P-P-lipid. As a consequence of this

glycosylation defect, this mutant tranfers oligosaccharides of a different structure than wild type to the lysosomal enzyme beta-hexosaminidase. In addition, it underglycosylates its proteins.

L3 ANSWER 27 OF 30 BIOSIS · COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 12

ACCESSION NUMBER: 1983:206859 BIOSIS
DOCUMENT NUMBER: BA75:56859
TITLE: A **CHINESE HAMSTER OVARY** CELL MUTANT DEFICIENT IN GLUCOSYLATION OF LIPID LINKED OLIGO SACCHARIDE SYNTHESIZES LYSOSOMAL ENZYMES OF ALTERED STRUCTURE AND FUNCTION.
AUTHOR(S): KRAG S S; ROBBINS A R
CORPORATE SOURCE: DEP. BIOCHEM., JOHNS HOPKINS UNIV. SCH. HYGIENE PUBLIC HEALTH, BALTIMORE, MD 21205.
SOURCE: J BIOL CHEM, (1982) 257 (14), 8424-8431.
CODEN: JBCHA3. ISSN: 0021-9258.
FILE SEGMENT: BA; OLD
LANGUAGE: English

AB B211 is a **Chinese hamster ovary** cell mutant previously characterized as deficient in the glucosylation of lipid-linked oligosaccharides. The activities of several acid hydrolases were decreased in extracts of B211; e.g. .alpha.-L-**iduronidase** and .beta.-hexosaminidase activities were 1 and 20%, respectively, of the activities measured in extracts of parental (WTB) cells. A revertant of B211, able to glucosylate lipid-linked oligosaccharides, exhibited hydrolase activity up to 30 times greater than that of B211. The activities of hydrolases secreted by mutant, revertant and parent were similar. Sodium dodecyl sulfate gel electrophoresis of .beta.-hexosaminidase isolated from secretions of B211 grown with [2-3H]mannose revealed several .alpha. and .beta. polypeptides, the MW values of which differed by .+-.5000 from those measured for the corresponding polypeptides from WTB. .alpha.-L-**Iduronidase** secreted by B211 had a MW approximately twice that measured for parental enzyme. The [3H]mannose-containing oligosaccharides of .beta.-hexosaminidase and .alpha.-L-**iduronidase** were analyzed by enzymatic digestion and gel filtration chromatography. Both enzymes isolated from secretions of WTB cells contained phosphorylated and simple high mannose oligosaccharides, as well as oligosaccharides resistant to cleavage by endo-.beta.-N-acetylglucosaminidase H; the proportion of [3H]mannose in the 3 classes of oligosaccharides differed significantly in the 2 enzymes. Neither .beta.-hexosaminidase nor .alpha.-L-**iduronidase** from B211 contained phosphorylated or high mannose oligosaccharides; all of the radioactivity was found in oligosaccharides of the complex type. Consistent with the absence of phosphorylated oligosaccharides, .beta.-hexosaminidase and .alpha.-L-**iduronidase** from B211 were not internalized via the mannose 6-phosphate receptor. While B211 failed to phosphorylate endogenous acid hydrolases, membrane preparations from the mutant did phosphorylate exogenously added .beta.-hexosaminidase.

L3 ANSWER 28 OF 30 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 13

ACCESSION NUMBER: 1982:200434 BIOSIS
DOCUMENT NUMBER: BA73:60418
TITLE: THE MANNOSE 6 PHOSPHATE RECEPTOR OF **CHINESE HAMSTER OVARY** CELLS COMPARTMENTALIZATION OF ACID HYDROLASES IN MUTANTS WITH ALTERED RECEPTORS.
AUTHOR(S): ROBBINS A R; MYEROWITZ R
CORPORATE SOURCE: GENET. BIOCHEM. BRANCH, NATL. INST. ARTHRITIS, METAB. DIG. DIS., BETHESDA, MD. 20205.
SOURCE: J BIOL CHEM, (1981) 256 (20), 10623-10627.
CODEN: JBCHA3. ISSN: 0021-9258.
FILE SEGMENT: BA; OLD

LANGUAGE: English

AB The localization of acid hydrolases was examined in **Chinese hamster ovary** cells with defective mannose 6-phosphate receptors; these mutants had been shown to exhibit reduced uptake and altered binding of exogenously added acid hydrolase. Cells were grown in the presence of [3H]mannose, .alpha.-L-**iduronidase** and .beta.-hexosaminidase were immunoprecipitated sequentially, electrophoresed on polyacrylamide gels containing sodium dodecyl sulfate and detected by fluorography. About 55% of the .alpha.-L-**iduronidase** and .beta.-hexosaminidase synthesized by the mutants in 12 h was found in the growth medium; parental cells secreted only .apprx. 15%. The mutants also secreted 2-6 times more .alpha.-mannosidase, .beta.-glucuronidase and .alpha.-L-fucosidase than the parent, as determined by measurements of enzyme activity. Intracellular levels of these enzymes were reduced in the mutants. The mutants secreted acid hydrolases in the precursor forms, within the cells these enzymes resided in lysosomes and were processed normally; thus, the mutants appeared aberrant only with respect to distribution of hydrolases between intracellular and extracellular compartments. [35S]methionine-labeled .beta.-hexosaminidase and .alpha.-L-**iduronidase** secreted by the mutants were taken up normally by both human fibroblasts and wild type **CHO** cells and this uptake was inhibited by mannose 6-phosphate. Thus, the elevated secretion of acid hydrolases was not due to alteration of the mannose 6-phosphate recognition marker on the enzymes but appears to result from alterations in the mannose 6-phosphate receptor.

L3 ANSWER 29 OF 30 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1981:101880 BIOSIS
DOCUMENT NUMBER: BR21:36876
TITLE: A DEFECT IN BIOSYNTHESIS OF OLIGO SACCHARIDE LIPID RESULTS IN ALTERATION OF LYSOSOMAL HYDROLASE STRUCTURE AND FUNCTION.
AUTHOR(S): KRAG S S; ROBBINS A R
CORPORATE SOURCE: JOHNS HOPKINS UNIV., BALTIMORE, MD 21205.
SOURCE: 72ND ANNUAL MEETING OF THE AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, ST. LOUIS, MO., USA, MAY 31-JUNE 4, 1981. FED PROC, (1981) 40 (6), 1861.
CODEN: FEPRA7. ISSN: 0014-9446.
FILE SEGMENT: BR; OLD
LANGUAGE: English

L3 ANSWER 30 OF 30 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 14
ACCESSION NUMBER: 1981:137472 BIOSIS
DOCUMENT NUMBER: BA71:7464
TITLE: P ISO THIO CYANATO PHENYL-6-PHOSPHO-ALPHA-D MANNO PYRANOSIDE COUPLED TO ALBUMIN A MODEL COMPOUND RECOGNIZED BY THE FIBROBLAST LYSOSOMAL ENZYME UPTAKE SYSTEM 2. BIOLOGICAL PROPERTIES.
AUTHOR(S): KARSON E M; NEUFELD E F; SANDO G N
CORPORATE SOURCE: DEP. INTERN. MED., UNIV. IOWA, IOWA CITY, IOWA 52242, USA.
SOURCE: BIOCHEMISTRY, (1980) 19 (16), 3856-3860.
CODEN: BICHAW. ISSN: 0006-2960.
FILE SEGMENT: BA; OLD
LANGUAGE: English

AB A conjugate of p-aminophenyl 6-phospho-.alpha.-D-mannopyranoside and bovine serum albumin interacted with the uptake system for lysosomal enzymes in cultured human diploid fibroblasts. Radioiodinated conjugate containing 20 mol of mannose 6-phosphate/mol of albumin was taken up by the cells and degraded to trichloroacetic acid soluble fragments which were released into the medium. Unlabeled conjugate, mannose 6-phosphate and a lysosomal enzyme, L-**iduronidase** (EC 3.2.1.76), inhibited the uptake of the 125I-labeled conjugate (Ki = 2 .times. 10-8, 5 .times. 10-6 and 1.5 .times. 10-9 M, respectively). The uptake of L-

iduronidase was competitively inhibited by the mannose 6-phosphate conjugate as well as by free mannose 6-phosphate; however, higher concentrations of these compounds were required (Ki = 10-6 and 5 .times. 10-5 M, respectively). Although L-**iduronidase** and the conjugate are bound to the same receptor by mannose 6-phosphate residues, the uptake of the enzyme involves additional structure that is not shared by the conjugate. Internalization of the radiolabeled mannose 6-phosphate albumin conjugate was observed only in human diploid fibroblast strains. An SV-40 transformed line of human fibroblasts as well as 3 permanent rodent fibroblast lines (**CHO** [**chinese hamster ovary** cell] NRK [rat kidney cell] and L-cells [mouse fibroblast cells]) failed to take up the conjugate, presumably because they were deficient in receptors or in the ability to internalize receptor-conjugate complexes.

=> d l3 ibib ab 1-24

L3 ANSWER 1 OF 30 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2003:408803 CAPLUS
TITLE: Large scale production and purification of human recombinant .alpha.-L-**iduronidase** for treating mucopolysaccharidosis I
INVENTOR(S): Qin, Minmin; Chan, Wai-pan; Chen, Lin; Fitzpatrick, Paul A.; Hendstrand, John M.; Wendt, Dan J.; Zecherle, Gary N.; Starr, Christopher M.; Kakkis, Emil D.
PATENT ASSIGNEE(S): Biomarin Pharmaceutical Inc., USA
SOURCE: U.S., 34 pp., Cont.-in-part of U.S. Ser. No. 439,923.
CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 4
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 6569661	B1	20030527	US 2000-711202	20001109
US 6426208	B1	20020730	US 1999-439923	19991112
WO 2002038775	A2	20020516	WO 2001-US47843	20011109
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
AU 2002027369	A5	20020521	AU 2002-27369	20011109
US 2002146802	A1	20021010	US 2001-993038	20011113
US 2002164758	A1	20021107	US 2001-993241	20011113
US 2003013179	A1	20030116	US 2002-206443	20020725

PRIORITY APPLN. INFO.:
US 1999-439923 A2 19991112
US 2000-711202 A 20001109
US 2000-711205 A1 20001109
WO 2001-US47843 W 20011109

AB The present invention provides a method to mass produce human recombinant .alpha.-L-**iduronidase** in large scale amts. with appropriate purity to enable large scale prodn. for long term patient use of the enzyme therapy. The method comprises the steps of: (a) harvesting and filtering fluid obtained from a culture of **Chinese hamster ovary** cells transformed with nucleic acids encoding the human recombinant .alpha.-L-**iduronidase**; (b) adjusting the pH of the fluid to an acidic pH wherein any potential virus

is inactivated and said human recombinant .alpha.-L-**iduronidase** is not harmed, followed by filtration through a 0.2 .mu. to 0.54 .mu. filter; (c) passing the fluid from step (b) through a Cibacron Blue dye interaction chromatog. column to capture the human recombinant .alpha.-L-**iduronidase**; (d) passing the fluid through a copper chelation chromatog. column to remove contaminating **Chinese hamster ovary** proteins; (e) passing the fluid through a Ph hydrophobic interaction chromatog. column to reduce residual leached Cibacron Blue dye and copper ions canned over from previous columns; and (f) concg. and diafiltering the purified human recombinant .alpha.-L-**iduronidase**; wherein said purity of equal to or greater than .apprx.99% purity of human recombinant .alpha.-L-**iduronidase** is measured by .mu.g of contaminating **Chinese hamster ovary** protein per mg of total protein. The purified com. grade recombinant human .alpha.-L-**iduronidase** can be used for treating genetic disorders including .alpha.-L-**iduronidase** deficiency and mucopolysaccharidosis I.

REFERENCE COUNT: 43 THERE ARE 43 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 2 OF 30 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 1

ACCESSION NUMBER: 2003:150962 BIOSIS
DOCUMENT NUMBER: PREV200300150962
TITLE: Identification and characterization of 13 new mutations in mucopolysaccharidosis type I patients.
AUTHOR(S): Matte, Ursula (1); Yogalingam, Gouri; Brooks, Doug; Leistner, Sandra; Schwartz, Ida; Lima, Luciane; Norato, Denise Y.; Brum, Jaime M.; Beesley, Clare; Winchester, Bryan; Giugliani, Roberto; Hopwood, John J.
CORPORATE SOURCE: (1) Medical Genetics Service, Hospital de Clinicas de Porto Alegre, Porto Alegre, RS, Brazil: matte@orion.ufrgs.br Brazil
SOURCE: Molecular Genetics and Metabolism, (January 2003, 2003) Vol. 78, No. 1, pp. 37-43. print. ISSN: 1096-7192.
DOCUMENT TYPE: Article
LANGUAGE: English

AB In this study we have investigated a group of 29 Brazilian patients, who had been diagnosed with the lysosomal storage disorder, Mucopolysaccharidosis type I (MPS-I). MPS I is caused by a deficiency in the lysosomal hydrolase, alpha-L-**iduronidase**. Ninety percent of the MPS I patients in this study were genotyped and revealed 10 recurrent and thirteen novel IDUA gene mutations. Eight of these new mutations and three common mutations W402X, P533R, and R383H were individually expressed in **CHO**-K1 cells and analyzed for alpha-L-**iduronidase** protein and enzyme activity. A correlation was observed between the MPS I patient clinical phenotype and the associated mutant alpha-L-**iduronidase** protein/enzyme activity expressed in **CHO**-K1 cells. This was the first time that Brazilian MPS I patients had been thoroughly analyzed and highlighted the difficulties of mutation screening and clinical phenotype assessment in populations with high numbers of unique mutations.

L3 ANSWER 3 OF 30 CAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 2002:368665 CAPLUS
DOCUMENT NUMBER: 136:385047
TITLE: Methods for large scale production and purification of human .alpha.-L-**iduronidase** for treatment of mucopolysaccharidosis I
INVENTOR(S): Qin, Minmin; Chan, Wai-Pan; Chen, Lin; Fitzpatrick, Paul A.; Henstrand, John M.; Wendt, Dan J.; Zecherle, Gary N.; Starr, Christopher M.; Kakkis, Emil D.
PATENT ASSIGNEE(S): Biomarin Pharmaceutical, Inc., USA

SOURCE: PCT Int. Appl., 71 pp.
CODEN: PIXXD2

DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 4
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002038775	A2	20020516	WO 2001-US47843	20011109
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
US 6569661	B1	20030527	US 2000-711202	20001109
AU 2002027369	A5	20020521	AU 2002-27369	20011109

PRIORITY APPLN. INFO.:
US 2000-711202 A 20001109
US 1999-439923 A2 19991112
WO 2001-US47843 W 20011109

AB The present invention provides a recombinant human .alpha.-L-**iduronidase** and biol. active fragments and muteins thereof with a purity greater than 99%. The present invention further provides large-scale methods to produce and purify com. grade recombinant human .alpha.-L-**iduronidase** enzyme thereof. The method involves prepn. of a seed culture contg. **Chinese hamster ovary** cells **2.131** transfected with a vector encoding cDNA for .alpha.-L-**iduronidase**. These cells is washed and resuspended in a protein-free culture medium supplemented with 7.6 mg/L thymidine, 13.6 mg/L hypoxanthine, 375 .mu.g/mL G418 and 5% fetal bovine serum. The cell suspension is incubated at 37.degree.C for 2-3 days with 5% CO2 in three 225 cm flasks. The said cell suspension is split by sequentially adding the cells to one 1L spinner flask, two 3L flasks and 4 8L flasks. The cell suspension is stirred at 50 rpm, followed by increasing the inoculum vol. by incubating and subculturing cells to a final cell d. of about 2-2.5 x 105. A mixt. contg. macroporous microcarriers is prepd. in growth medium with fetal bovine serum and transfering said mixt. to a bioreactor. Cells from the bioreactor may be harvested at a d. of about 106. Methods for purifn. of .alpha.-L-**iduronidase** to greater than 99% purity include adjusting the pH to an acidic range, followed by filtering the mixt. through a 0.2-0.54 .mu. filter. The filtrate is further passed through a blue sepharose FF column to capture the protein which purifies .alpha.-L-**iduronidase** 7-10-fold. Contaminating **CHO** proteins are removed by passing the fluid through a copper chelating sepharose column. The mixt. is then passed through a Ph sepharose column to reduce residual leached Cibacron blue dye and copper ions carried over from the previous columns. Purified .alpha.-L-**iduronidase** is concd. and diafiltered. The purifn. steps include 10% glycerol in all buffers to improve the .alpha.-L-**iduronidase** yield. The specific activity of .alpha.-L-**iduronidase** may be greater than 240,000 units/mg protein.

L3 ANSWER 4 OF 30 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2002:51616 CAPLUS
DOCUMENT NUMBER: 136:98430
TITLE: Large scale recombinant .alpha.-L-**iduronidase** production and purification for mucopolysaccharidosis I enzyme-replacement therapy
INVENTOR(S): Hendstrand, John M.; Qin, Minmin; Chan, Wia-Pan; Chen, Lin; Fitzpatrick, Paul A.; Wendt, Dan J.; Zecherle,

Gary N.; Starr, Christopher M.; Kakkis, Emil D.

PATENT ASSIGNEE(S): Biomarin Pharmaceuticals, USA; Harbor-UCLA Research and Education Institute

SOURCE: PCT Int. Appl., 67 pp.
CODEN: PIXXD2

DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 4
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002004616	A1	20020117	WO 2000-US31293	20001109
W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
US 6426208	B1	20020730	US 1999-439923	19991112
BR 2000015533	A	20020625	BR 2000-15533	20001109
US 2002164758	A1	20021107	US 2001-993241	20011113
US 2003013179	A1	20030116	US 2002-206443	20020725

PRIORITY APPLN. INFO.:

US 1999-439923	A	19991112
US 2000-711205	A1	20001109
WO 2000-US31293	W	20001109

AB In one aspect, the present invention features a method to mass produce human recombinant .alpha.-L-**iduronidase** in large scale amts. with appropriate purity to enable large scale prodn. for long term patient use of the enzyme therapy. In a broad embodiment, the method comprises the step of transfecting a cDNA encoding for all or part of an .alpha.-L-**iduronidase** into a cell suitable for the expression thereof. In one particularly preferred embodiment, the cDNA is transfected into a **Chinese hamster ovary** cell to create cell line **2.131**. In yet other preferred embodiments, the prodn. procedure features one or more of the following characteristics which have demonstrated particularly high prodn. levels: (a) the pH of the cell growth culture may be lowered to about 6.5 to 7.0, preferably to about 6.8-7.0 during the prodn. process, (b) as many as 2 to 3.5 culture vols. of the medium may be changed during each 24-h period by continuous perfusion, (c) oxygen satn. may be optimized to about 40 % but may be as high as 80 %, (d) macroporous cellulose microcarriers with about 5 % serum in the medium initially, may be used to produce cell mass followed by a rapid washout shift to protein-free medium for prodn., (e) a protein-free or low protein-medium such as a JRH Biosciences PF-**CHO** product may be optimized to include supplemental amts. of one or more ingredients selected from the group consisting of: glutamate, aspartate, glycine, ribonucleosides, and deoxyribonucleosides; (f) a stirred tank suspension culture may be perfused in a continuous process to produce **iduronidase**. In one preferred embodiment, a three step column chromatog. may be used to purify the enzyme. Such a three step column chromatog. may include using a blue sepharose FF, a Cu++ chelating sepharose chromatog. and a Ph sepharose HP chromatog. In another preferred embodiment, an acid pH treatment step is used to inactivate potential viruses without harming the enzyme. In another aspect, the present invention features novel methods of treating diseases caused all or in part by a deficiency in .alpha.-L-**iduronidase**. In one embodiment, this method features administering a recombinant .alpha.-L-**iduronidase** or a biol. active fragment or mutant thereof alone or in combination with a pharmaceutically suitable carrier. In preferred embodiments, the disease is Mucopolysaccharidosis I (MPS I), Hurler

syndrome, Hurler-Scheie syndrome or Scheie syndrome. Mucopolysaccharidosis I is a lysosomal storage disease caused by a deficiency of the enzyme .alpha.-L-**iduronidase**. We evaluated the effect of enzyme-replacement therapy with recombinant human .alpha.-L-**iduronidase** in patients with this disorder. We treated 10 patients with mucopolysaccharidosis I (age, 5 to 22 yr) with recombinant human .alpha.-L-**iduronidase** at a dose of 125,000 U per kg of body wt. given i.v. once weekly for 52 wk. The patients were evaluated at base line and at 6, 12, 26, and 52 wk by detailed clin. examns., magnetic resonance imaging of the abdomen and brain, echocardiog., range-of-motion measurements, polysomnog., clin. lab. evaluations, measurements of leukocyte .alpha.-L-**iduronidase** activity, and urinary glycosaminoglycan excretion. Hepatosplenomegaly decreased significantly in all patients, and the size of the liver was normal for body wt. and age in eight patients by 26 wk. The rate of growth in height and wt. had increased by a mean of 85 and 131 %, resp., at 52 wk in the six prepubertal patients. The mean maximal range of motion of shoulder flexion and elbow extension increased significantly. The no. of episodes of apnea and hypopnea during sleep decreased 61 %. New York Heart Assocn. functional class improved by one or two classes in all patients. Urinary glycosaminoglycan excretion decreased after three to four weeks of treatment; the mean redn. at 52 wk was 63 % of base-line values. Five patients had transient urticaria during infusions. Serum antibodies to .alpha.-L-**iduronidase** were detected in four patients. In conclusion, in patients with mucopolysaccharidosis I, treatment with recombinant human .alpha.-L-**iduronidase** reduces lysosomal storage in the liver and ameliorates some clin. manifestations of the disease.

REFERENCE COUNT: 6 THERE ARE 6 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 5 OF 30 PROMT COPYRIGHT 2003 Gale Group

ACCESSION NUMBER: 2001:315156 PROMT
TITLE: Glyko Biomedical Ltd.'s 30.6%-owned affiliate, BioMarin Completes Important Milestone in the Manufacturing of Aldurazyme.
SOURCE: Business Wire, (24 Apr 2001) pp. 375.
PUBLISHER: Business Wire
DOCUMENT TYPE: Newsletter
LANGUAGE: English
WORD COUNT: 2704
FULL TEXT IS AVAILABLE IN THE ALL FORMAT

AB Business/Health Editors
THIS IS THE FULL TEXT: COPYRIGHT 2001 Business Wire

L3 ANSWER 6 OF 30 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2001:507831 CAPLUS
DOCUMENT NUMBER: 135:118780
TITLE: Improved lysosomal enzymes and lysosomal enzyme activators containing additional glycosylation sites introduced by site-specific mutagenesis
INVENTOR(S): Okkels, Jens Sigurd; Jensen, Anne Dam; Halkier, Torben; Jensen, Rikke Bolding; Schambye, Hans Thalsgaard
PATENT ASSIGNEE(S): Maxygen Aps, Den.
SOURCE: PCT Int. Appl., 98 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE

WO 2001049830	A2	20010712	WO 2000-DK743	20001229
WO 2001049830	A3	20020207		
WO 2001049830	C2	20021107		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
US 2002127219	A1	20020912	US 2000-753126	20001229
EP 1246915	A2	20021009	EP 2000-987208	20001229
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				
WO 2002002597.	A2	20020110	WO 2001-DK459	20010629
WO 2002002597	A3	20020627		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
US 2003036181	A1	20030220	US 2001-896896	20010629
EP 1299535	A2	20030409	EP 2001-944987	20010629
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				

PRIORITY APPLN. INFO.:	DK 1999-1891	A 19991230
	DK 2000-865	A 20000602
	DK 2000-866	A 20000602
	DK 2000-1027	A 20000630
	US 2000-174652P	P 20000106
	US 2000-210984P	P 20000612
	US 2000-211124P	P 20000612
	US 2000-217497P	P 20000711
	DK 2000-1092	A 20000714
	US 2000-225558P	P 20000816
	WO 2000-DK743	W 20001229
	WO 2001-DK90	W 20010209
	WO 2001-DK459	W 20010629

AB A polypeptide selected from the group of lysosomal enzymes and lysosomal enzyme activators, comprising at least one introduced glycosylation site as compared to a corresponding parent enzyme or activator. By introducing addnl. glycosylation sites the resulting glycosylated lysosomal enzyme or activator obtains improved in vivo activity and thereby provides for improved treatment of lysosomal storage diseases. Thus, in particular, glycosylation sites (Ser or Thr residues) are introduced into human glucocerebrosidase, and esp. in peptide addns. to the N-terminus of glucocerebrosidase. A fusion protein was also constructed comprising saposin C, a linker peptide, and human glucocerebrosidase. The N-glycan structures in glucocerebrosidase and its variants expressed in insect cells are characterized.

L3 ANSWER 7 OF 30 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 2

ACCESSION NUMBER: 2001:492293 BIOSIS
DOCUMENT NUMBER: PREV200100492293
TITLE: Glycosidase active site mutations in human alpha-L-**iduronidase**.
AUTHOR(S): Brooks, Doug A. (1); Fabrega, Sylvie; Hein, Leanne K.;

Parkinson, Emma J.; Durand, Patrick; Yogalingam, Gouri; Matte, Ursula; Giugliani, Roberto; Dasvarma, Ayan; Eslahpazire, Jobin; Henrissat, Bernard; Mornon, Jean-Paul; Hopwood, John J.; Lehn, Pierre

CORPORATE SOURCE: (1) Lysosomal Diseases Research Unit, Department of Chemical Pathology, Women's and Children's Hospital, King William Road, North Adelaide, SA, 5006 Australia

SOURCE: Glycobiology, (September, 2001) Vol. 11, No. 9, pp. 741-750. print.
ISSN: 0959-6658.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Mucopolysaccharidosis type I (MPS I; McKusick 25280) results from a deficiency in alpha-L-**iduronidase** activity. Using a bioinformatics approach, we have previously predicted the putative acid/base catalyst and nucleophile residues in the active site of this human lysosomal glycosidase to be Glu182 and Glu299, respectively. To obtain experimental evidence supporting these predictions, wild-type alpha-L-**iduronidase** and site-directed mutants E182A and E299A were individually expressed in **Chinese hamster ovary**-K1 cell lines. We have compared the synthesis, processing, and catalytic properties of the two mutant proteins with wild-type human alpha-L-**iduronidase**. Both E182A and E299A transfected cells produced catalytically inactive human alpha-L-**iduronidase** protein at levels comparable to the wild-type control. The E182A protein was synthesized, processed, targeted to the lysosome, and secreted in a similar fashion to wild-type alpha-L-**iduronidase**. The E299A mutant protein was also synthesized and secreted similarly to the wild-type enzyme, but there were alterations in its rate of traffic and proteolytic processing. These data indicate that the enzymatic inactivity of the E182A and E299A mutants is not due to problems of synthesis/folding, but to the removal of key catalytic residues. In addition, we have identified a MPS I patient with an E182K mutant allele. The E182K mutant protein was expressed in **CHO**-K1 cells and also found to be enzymatically inactive. Together, these results support the predicted role of E182 and E299 in the catalytic mechanism of alpha-L-**iduronidase** and we propose that the mutation of either of these residues would contribute to a very severe clinical phenotype in a MPS I patient.

L3 ANSWER 8 OF 30 PROMT COPYRIGHT 2003 Gale Group

ACCESSION NUMBER: 2000:904534 PROMT
TITLE: Genzyme Corp. Announces Third-Quarter Financial Results for Genzyme General.
SOURCE: PR Newswire, (19 Oct 2000) .
PUBLISHER: PR Newswire Association, Inc.
DOCUMENT TYPE: Newsletter
LANGUAGE: English
WORD COUNT: 4785

FULL TEXT IS AVAILABLE IN THE ALL FORMAT

AB Strong Earnings and Revenue Growth Reported

L3 ANSWER 9 OF 30 MEDLINE DUPLICATE 3

ACCESSION NUMBER: 2000207139 MEDLINE
DOCUMENT NUMBER: 20207139 PubMed ID: 10739940

TITLE: alpha-L-**iduronidase** forms semi-crystalline spherulites with amyloid-like properties.

AUTHOR: Ruth L; Eisenberg D; Neufeld E F
CORPORATE SOURCE: Department of Biological Chemistry and Molecular Biology Institute, University California Los Angeles, Los Angeles, CA 90095, USA.

CONTRACT NUMBER: GM08243 (NIGMS)
SOURCE: ACTA CRYSTALLOGRAPHICA. SECTION D: BIOLOGICAL CRYSTALLOGRAPHY, (2000 Apr) 56 (Pt 4) 524-8. Journal code: 9305878. ISSN: 0907-4449.
PUB. COUNTRY: Denmark
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200006
ENTRY DATE: Entered STN: 20000616
Last Updated on STN: 20000616
Entered Medline: 20000605

AB While seeking conditions for single crystals of human alpha-L-**iduronidase**, solutions were discovered (pH 3.0-8.5 containing calcium or zinc salts) that transform soluble alpha-L-**iduronidase** to a solid aggregate. This aggregate is a spherulite of semi-crystalline protein. The X-ray diffraction pattern and ability to bind Congo red characterize the alpha-L-**iduronidase** spherulite as 'amyloid-like', in that it displays two of the characteristics of amyloidogenic proteins. In addition, alpha-L-**iduronidase** also interacts with heparin, as do some amyloid-forming proteins.

L3 ANSWER 10 OF 30 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 4
ACCESSION NUMBER: 2000:386539 BIOSIS
DOCUMENT NUMBER: PREV200000386539
TITLE: Purification and characterization of recombinant human alpha-N-acetylglucosaminidase secreted by **Chinese hamster ovary** cells.
AUTHOR(S): Zhao, Ke-Wei; Neufeld, Elizabeth F. (1)
CORPORATE SOURCE: (1) Department of Biological Chemistry, UCLA School of Medicine, 10833 Le Conte Avenue, 33-257 CHS, Los Angeles, CA, 90095-1737 USA
SOURCE: Protein Expression and Purification, (June, 2000) Vol. 19, No. 1, pp. 202-211. print.
ISSN: 1046-5928.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English

AB alpha-N-Acetylglucosaminidase (EC 3.2.1.50) is a lysosomal enzyme that is deficient in the genetic disorder Sanfilippo syndrome type B. To study the human enzyme, we expressed its cDNA in Lec1 mutant **Chinese hamster ovary** (**CHO**) cells, which do not synthesize complex oligosaccharides. The enzyme was purified to apparent homogeneity from culture medium by chromatography on concanavalin A-Sepharose, Poros 20-heparin, and aminooctyl-agarose. The purified enzyme migrated as a single band of 83 kDa on SDS-PAGE and as two peaks corresponding to monomeric and dimeric forms on Sephacryl-300. It had an apparent Km of 0.22 mM toward 4-methylumbelliferyl-alpha-N-acetylglucosaminide and was competitively inhibited by two potential transition analogs, 2-acetamido-1,2-dideoxynojirimycin (Ki = 0.45 muM) and 6-acetamido-6-deoxycastanospermine (Ki = 0.087 muM). Activity was also inhibited by mercurials but not by N-ethylmaleimide or iodoacetamide, suggesting the presence of essential sulfhydryl residues that are buried. The purified enzyme preparation corrected the abnormal (35S)glycosaminoglycan catabolism of Sanfilippo B fibroblasts in a mannose 6-phosphate-inhibitable manner, but its effectiveness was surprisingly low. Metabolic labeling experiments showed that the recombinant alpha-N-acetylglucosaminidase secreted by **CHO** cells had only a trace of mannose 6-phosphate, probably derived from contaminating endogenous **CHO** enzyme. This contrasts with the presence of mannose 6-phosphate on naturally occurring alpha-N-acetylglucosaminidase secreted by diploid human fibroblasts and on recombinant human alpha-L-**iduronidase** secreted by the same **CHO** cells. Thus

contrary to current belief, overexpressing **CHO** cells do not necessarily secrete recombinant lysosomal enzyme with the mannose 6-phosphate-targeting signal; this finding has implications for the preparation of such enzymes for therapeutic purposes.

L3 ANSWER 11 OF 30 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1999:736948 CAPLUS
DOCUMENT NUMBER: 131:348539
TITLE: Recombinant human (alpha)-l-**iduronidase**, methods for producing and purifying the same and methods for treating diseases caused by deficiencies thereof
INVENTOR(S): Kakkis, Emil D.; Tanamachi, Becky
PATENT ASSIGNEE(S): Harbor-UCLA, USA
SOURCE: PCT Int. Appl., 35 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9958691	A2	19991118	WO 1999-US10102	19990507
WO 9958691	A3	20000217		
W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
CA 2328518	AA	19991118	CA 1999-2328518	19990507
AU 9940724	A1	19991129	AU 1999-40724	19990507
BR 9910323	A	20010130	BR 1999-10323	19990507
EP 1078075	A2	20010228	EP 1999-924155	19990507
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
JP 2002514429	T2	20020521	JP 2000-548482	19990507

PRIORITY APPLN. INFO.:
US 1998-78209 A 19980513
US 1998-170977 A 19981013
WO 1999-US10102 W 19990507

AB The present invention provides a recombinant .alpha.-L-**iduronidase** and biol. active fragments and mutants thereof, methods to produce and purify this enzyme as well as methods to treat certain genetic disorders including .alpha.-L-**iduronidase** deficiency and mucopolysaccharidosis I (MPS I). The authors demonstrate in three patients substantial shrinkage of liver and spleen with significant clin. improvement in joint and soft tissue storage assocd. with greater than 65% redn. of undegraded GAG after only 8 wk treatment with the recombinant enzyme. This enzyme was manufd. and secreted from **CHO** cells growth in microcarriers where the pH is lowered to 6.7-6.8. Most of the growth medium is changed every 12 h and the oxygen satn. is optimized at 80% by intermittent pure oxygen sparging. A washout shift to protein-free medium for protein secretion and prodn. is employed. JRH Biosciences PF-**CHO** growth medium is used. The growth medium is optimized to include supplemental amts. of one or more ingredients selected from the group consisting of glutamate, aspartate, glycine, ribonucleosides and deoxyribonucleosides. Here, a batch-fed process is performed by a perfusion wand. Sodium butyrate is added to the medium also. Purifn. methods involve concn., diafiltration and acidification and running the sample on heparin, Ph and Sephacryl columns. Hurler's disease, Scheie

syndrome and Hurler-scheie syndromes are all treated.

L3 ANSWER 12 OF 30 SCISEARCH COPYRIGHT 2003 THOMSON ISI
ACCESSION NUMBER: 1998:430904 SCISEARCH
THE GENUINE ARTICLE: ZQ947
TITLE: Mild feline mucopolysaccharidosis type VI - Identification of an N-acetylgalactosamine-4-sulfatase mutation causing instability and increased specific activity
AUTHOR: Yogalingam G; Hopwood J J; Crawley A; Anson D S (Reprint)
CORPORATE SOURCE: WOMENS & CHILDRENS HOSP, DEPT CHEM PATHOL, LYSOSOMAL DIS RES UNIT, 72 KING WILLIAM RD, N ADELAIDE, SA 5006, AUSTRALIA (Reprint); WOMENS & CHILDRENS HOSP, DEPT CHEM PATHOL, LYSOSOMAL DIS RES UNIT, N ADELAIDE, SA 5006, AUSTRALIA
COUNTRY OF AUTHOR: AUSTRALIA
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (29 MAY 1998) Vol. 273, No. 22, pp. 13421-13429.
Publisher: AMER SOC BIOCHEMISTRY MOLECULAR BIOLOGY INC, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814.
ISSN: 0021-9258.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 25
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The missense mutation, L476P, in the N-acetylgalactosamine 4-sulfatase (4S) gene, has previously been shown to be associated with a severe feline mucopolysaccharidosis type VI (MPS VI) phenotype, The present study describes a second mutation, D520N, in the same MPS VI cat colony, which is inherited independently of L476P and is associated with a clinically mild MPS VI phenotype in D520N/L476P compound heterozygous cats. Biochemical and clinical assessment of L476P homozygous, D520N/L476P compound heterozygous, and D520N homozygous cats demonstrated that the entire range of clinical phenotypes, from severe MPS VI, to mild MPS VI, to normal are clustered within a narrow range of residual 4S activity from 0.5% to 4.6% of normal levels. When overexpressed in **CHO**-KI cells, the secreted form of D520N 4S was inactivated in neutral pH conditions. In addition, intracellular D520N 4S protein was rapidly degraded and corresponded to 37%, 14.5%, and 0.67% of normal 4S protein levels in the microsomal, endosomal, and lysosomal compartments, respectively. However, the specific activity of lysosomal D520N 4S was elevated 22.5-fold when compared with wild-type 4S, These results suggest that the D520N mutation causes a rapid degradation of 4S protein, The effect of this is partially ameliorated as a result of a significant elevation in the specific activity of mutant D520N 4S reaching the lysosomal compartment.

L3 ANSWER 13 OF 30 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 5
ACCESSION NUMBER: 1999:35655 BIOSIS
DOCUMENT NUMBER: PREV199900035655
TITLE: Structural determination of oligosaccharides from recombinant **iduronidase** released with peptide N-glycanase F using fluorophore-assisted carbohydrate electrophoresis.
AUTHOR(S): Hague, Chuck; Masada, R. Irene; Starr, Christopher (1)
CORPORATE SOURCE: (1) Glyko Inc., 11 Pimentel Ct., Novato, CA 94949 USA
SOURCE: Electrophoresis, (Nov., 1998) Vol. 19, No. 15, pp. 2612-2620.
ISSN: 0173-0835.
DOCUMENT TYPE: Article
LANGUAGE: English

AB The lysosomal storage disorder mucopolysaccharidoses I (MPS I) is caused by a deficiency in the production of alpha-L-**iduronidase**.

Recently, a recombinant alpha-L-**iduronidase** has been produced in **Chinese hamster ovary** (**CHO**) cells.
It is thought that for alpha-L-**iduronidase** to be correctly targeted to the lysosomal vesicle a particular oligosaccharide make-up must be present, and characterization of the carbohydrates is critical. Oligosaccharides from alpha-L-**iduronidase** were analyzed using fluorophore-assisted carbohydrate electrophoresis (FACE). The FACE' system uses polyacrylamide gel electrophoresis to separate, quantify, and determine the sequence of oligosaccharides released from glycoproteins. Asparagine-linked oligosaccharides were released from alpha-L-**iduronidase** using the enzyme peptide N-glycanase F (PNGase F). Released oligosaccharides were labeled with a fluorophore at-the reducing termini by reductive amination. A total of nine bands were sequenced from the released pool of oligosaccharides. The pool of fluorescently labeled oligosaccharides was then electrophoresed in preparative gels and each band individually excised and extracted. Isolated bands were treated with a series of exoenzymes to determine the sequence of monosaccharides that make up a particular oligosaccharide. A total of eighteen different oligosaccharides were identified from the original pool of oligosaccharides. A majority of the oligosaccharides, over 73%, were found to be of the sialylated complex type. Four of the oligosaccharides were phosphorylated, making up approximately 11% of the carbohydrate pool, and the remaining 15% were of the oligomannose type.

L3 ANSWER 14 OF 30 SCISEARCH COPYRIGHT 2003 THOMSON ISI
ACCESSION NUMBER: 1998:788927 SCISEARCH
THE GENUINE ARTICLE: 126RY
TITLE: Recombinant human acid alpha-glucosidase: high level production in mouse milk, biochemical characteristics, correction of enzyme deficiency in GSDII KO mice
AUTHOR: Bijvoet A G A; Kroos M A; Pieper F R; VanderVliet M; DeBoer H A; VanderPloeg A T; Verbeet M P; Reuser A J J (Reprint)
CORPORATE SOURCE: ERASMUS UNIV, DEPT CLIN GENET, POB 1738, NL-3000 DR ROTTERDAM, NETHERLANDS (Reprint); ERASMUS UNIV, DEPT CLIN GENET, NL-3000 DR ROTTERDAM, NETHERLANDS; SOPHIA CHILDRENS UNIV HOSP, DEPT PAEDIAT, NL-3000 CB ROTTERDAM, NETHERLANDS; PHARMING BV, NL-2333 CA LEIDEN, NETHERLANDS; BIOCELL TECHNOL, NL-2207 CK SPIJKENISSE, NETHERLANDS; LEIDEN UNIV, LEIDEN INST CHEM, METALLOPROT & PROT ENGN GRP, NL-2300 RA LEIDEN, NETHERLANDS
COUNTRY OF AUTHOR: NETHERLANDS
SOURCE: HUMAN MOLECULAR GENETICS, (OCT 1998) Vol. 7, No. 11, pp. 1815-1824.
Publisher: OXFORD UNIV PRESS, GREAT CLARENDON ST, OXFORD OX2 6DP, ENGLAND.
ISSN: 0964-6906.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 52

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Glycogen storage disease type II (GSDII) is caused by lysosomal acid alpha-glucosidase deficiency. Patients have a rapidly fatal or slowly progressive impairment of muscle function. Enzyme replacement therapy is under investigation, For large-scale, cost-effective production of recombinant human acid alpha-glucosidase in the milk of transgenic animals, we have fused the human acid alpha-glucosidase gene to 6.3 kb of the bovine alpha(S1)-casein gene promoter and have tested the performance of this transgene in mice. The highest production level reached was 2 mg/ml, The major fraction of the purified recombinant enzyme has a molecular mass of 110 kDa and resembles the natural acid alpha-glucosidase precursor from human urine and the recombinant precursor secreted by **CHO** cells, with respect to pH optimum, K-m, V-max, N-terminal

amino acid sequence and glycosylation pattern. The therapeutic potential of the recombinant enzyme produced in milk is demonstrated in vitro and in vivo, The precursor is taken up in a mannose 6-phosphate receptor-dependent manner by cultured fibroblasts, is converted to mature enzyme of 76 kDa and depletes the glycogen deposit in fibroblasts of patients. When injected intravenously, the milk enzyme corrects the acid alpha-glucosidase deficiency in heart and skeletal muscle of GSDII knockout mice.

L3 ANSWER 15 OF 30 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 6

ACCESSION NUMBER: 1997:452113 BIOSIS
DOCUMENT NUMBER: PREV199799751316
TITLE: Carbohydrate structures of recombinant human alpha-L-**iduronidase** secreted by **Chinese hamster ovary** cells.
AUTHOR(S): Zhao, Ke-Wei; Faull, Kym F.; Kakkis, Emil D.; Neufeld, Elizabeth F. (1)
CORPORATE SOURCE: (1) Dep. Biol. Chem., UCLA Sch. Med., Los Angeles, CA 90095-1737 USA
SOURCE: Journal of Biological Chemistry, (1997) Vol. 272, No. 36, pp. 22758-22765.
ISSN: 0021-9258.
DOCUMENT TYPE: Article
LANGUAGE: English

AB alpha-L-**Iduronidase** is a lysosomal hydrolase that is deficient in Hurler syndrome and clinically milder variants. Recombinant human alpha-L-**iduronidase**, isolated from secretions of an overexpressing **Chinese hamster ovary** cell line, is potentially useful for replacement therapy of these disorders. Because of the importance of carbohydrate residues for endocytosis and lysosomal targeting, we examined the oligosaccharides of recombinant alpha-L-**iduronidase** at each of its six N-glycosylation sites. Biosynthetic radiolabeling showed that three or four of the six oligosaccharides of the secreted enzyme were cleaved by endo-beta-N-acetylglucosaminidase H, with phosphate present on the sensitive oligosaccharides and L-fucose on the resistant ones. For structural analysis, tryptic and chymotryptic glycopeptides were isolated by lectin binding and reverse phase high pressure liquid chromatography; their molecular mass was determined by matrix-assisted laser desorption-time of flight mass spectrometry before and after treatment with endo- or exoglycosidases or with alkaline phosphatase. Identification of the peptides was assisted by amino- or carboxyl-terminal sequence analysis. The major oligosaccharide structures found at each site were as follows: Asn110, complex; Asn-190, complex; Asn-336, bisphosphorylated (P-2Man-7GlcNAc-2); Asn-372, high mannose (mainly Man-9GlcNAc-2, some of which was monoglucosylated); Asn-415, mixed high mannose and complex; Asn451, bisphosphorylated (P-2Man7GlcNAc-2). The Asn451 glycopeptide was unexpectedly resistant to digestion by N-glycanase unless first dephosphorylated, but it was sensitive to endo-beta-N-acetylglucosaminidase H and to glycopeptidase A. The heterogeneity of carbohydrate structures must represent the accessibility of the glycosylation sites as well as the processing capability of the overexpressing **Chinese hamster ovary** cells.

L3 ANSWER 16 OF 30 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.

ACCESSION NUMBER: 1996:308095 BIOSIS
DOCUMENT NUMBER: PREV199699030451
TITLE: Oligosaccharides of recombinant human alpha-L-**iduronidase** secreted by **Chinese hamster ovary** cells.
AUTHOR(S): Zhao, K. W. (1); Stevens, R. L.; Faull, K. F.; Kakkis, E. D.; Neufeld, E. F.
CORPORATE SOURCE: (1) UCLA Sch. Med., Los Angeles, CA 90095 USA

SOURCE: FASEB Journal, (1996) Vol. 10; No. 6, pp. A1108.
Meeting Info.: Joint Meeting of the American Society for Biochemistry and Molecular Biology, the American Society for Investigative Pathology and the American Association of Immunologists New Orleans, Louisiana, USA June 2-6, 1996
ISSN: 0892-6638.
DOCUMENT TYPE: Conference
LANGUAGE: English

L3 ANSWER 17 OF 30 SCISEARCH COPYRIGHT 2003 THOMSON ISI
ACCESSION NUMBER: 96:397892 SCISEARCH
THE GENUINE ARTICLE: UK861
TITLE: OLIGOSACCHARIDES OF RECOMBINANT HUMAN ALPHA-L-**IDURONIDASE** SECRETED BY **CHINESE-HAMSTER OVARY** CELLS
AUTHOR: ZHAO K W (Reprint); STEVENS R L; FAULL K F; KAKKIS E D; NEUFELD E F
CORPORATE SOURCE: UNIV CALIF LOS ANGELES, SCH MED, LOS ANGELES, CA, 90095; UNIV CALIF LOS ANGELES, HARBOR MED CTR, TORRANCE, CA, 90502
COUNTRY OF AUTHOR: USA
SOURCE: FASEB JOURNAL, (30 APR 1996) Vol. 10, No. 6, pp. 631.
ISSN: 0892-6638.
DOCUMENT TYPE: Conference; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: No References

L3 ANSWER 18 OF 30 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1997:95098 BIOSIS
DOCUMENT NUMBER: PREV199799394301
TITLE: Selective association of overexpressed secreted lysosomal enzyme hydrolases with calnexin.
AUTHOR(S): Wilson, D.; Hechtman, P.; Kaplan, F.; Thomas, D. Y.; Bergeron, J. J. M.
CORPORATE SOURCE: McGill Univ., Biotechnology Res. Inst., Montreal, PQ Canada
SOURCE: Molecular Biology of the Cell, (1996) Vol. 7, No. SUPPL., pp. 136A.
Meeting Info.: Annual Meeting of the 6th International Congress on Cell Biology and the 36th American Society for Cell Biology San Francisco, California, USA December 7-11, 1996
ISSN: 1059-1524.
DOCUMENT TYPE: Conference; Abstract; Conference
LANGUAGE: English

L3 ANSWER 19 OF 30 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 7
ACCESSION NUMBER: 1995:63136 BIOSIS
DOCUMENT NUMBER: PREV199598077436
TITLE: Enzyme replacement in a canine model of Hurler syndrome.
AUTHOR(S): Shull, Robert M.; Kakkis, Emil D.; McEntee, Michael F.; Kania, Stephen A.; Jonas, Adam J.; Neufeld, Elizabeth F. (1)
CORPORATE SOURCE: (1) Dep. Biol. Chem., University California Sch. Med., Los Angeles, CA 90024-1737 USA
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (1994) Vol. 91, No. 26, pp. 12937-12941.
ISSN: 0027-8424.
DOCUMENT TYPE: Article
LANGUAGE: English
AB The Hurler syndrome (alpha-L-**iduronidase** deficiency disease) is a severe lysosomal storage disorder that is potentially amenable to

enzyme-replacement therapy. Availability of a canine model of the disease and a sufficient supply of corrective enzyme have permitted a therapeutic trial lasting 3 mo. Recombinant human alpha-L-**iduronidase**, purified to apparent homogeneity from secretions of a stably transfected **Chinese hamster ovary** cell line, was administered i.v. to homozygous affected animals in doses of apprxeq 1 mg. The enzyme rapidly disappeared from the circulation in a biphasic manner, with t-1/2 of 0.9 and 19 min, respectively, and was taken up primarily by the liver. Biopsy of the liver before and after a very short trial (seven doses administered over 12 days) showed remarkable resolution of lysosomal storage in both hepatocytes and Kupffer cells. After weekly administration of enzyme to three affected animals over a period of 3 mo, the level of enzyme was about normal in liver and spleen, lower but significant in kidney and lung, and barely detectable (0-5% of normal) in brain, heart valves, myocardium, cartilage, and cornea. Light and electron microscopic examination of numerous tissues showed normalization of lysosomal storage in liver, spleen, and kidney glomeruli, but there was no improvement in brain, heart valves, or cornea. Even though the treated dogs developed complement-activating antibodies against alpha-L-**iduronidase**, clinical symptoms could be prevented by slow infusion of enzyme and premedication.

L3 ANSWER 20 OF 30 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 8

ACCESSION NUMBER: 1994:345081 BIOSIS
DOCUMENT NUMBER: PREV199497358081
TITLE: Overexpression of the human lysosomal enzyme alpha-L-**iduronidase** in **Chinese hamster ovary** cells.
AUTHOR(S): Kakkis, Emil D. (1); Matynia, Anna; Jonas, Adam J.; Neufeld, Elizabeth F.
CORPORATE SOURCE: (1) Div. Med. Genetics, Harbor-UCLA Med. Cent., 1124 W. Carson St., Torrance, CA 90502 USA
SOURCE: Protein Expression and Purification, (1994) Vol. 5, No. 3, pp. 225-232.
ISSN: 1046-5928.
DOCUMENT TYPE: Article
LANGUAGE: English

AB We developed a **Chinese hamster ovary** (**CHO**) cell line that produces and secretes large quantities of recombinant human alpha-L-**iduronidase**, the lysosomal hydrolase deficient in mucopolysaccharidosis I (Hurler, Hurler-Scheie, and Scheie syndromes). The alpha-L-**iduronidase** cDNA was introduced into a vector containing the cytomegalovirus immediate early gene promoter/enhancer, a murine immunoglobulin C-alpha region intron, and the bovine growth hormone polyadenylation signal. Following cotransfection with a plasmid containing the neomycin resistance gene, stably transfected lines were selected with G-418. The highest expressing **CHO** cell line contained 1400-6000 units of alpha-L-**iduronidase** per milligram of protein, or 0.6-2.4% of total cell protein. Secreted alpha-L-**iduronidase** was 3000- to 7000 fold increased, with about 5000 units accumulating in 24 h per 10-7 cells. The activity and distribution of five other lysosomal glycosidases were not significantly affected. Metabolic labeling showed that half of the newly synthesized alpha-L-**iduronidase** was secreted, but generally less was recovered due to its instability in the medium. It was post-translationally processed as previously shown for alpha-L-**iduronidase** of human fibroblasts. Recombinant alpha-L-**iduronidase** was efficiently endocytosed by Hurler fibroblasts utilizing a mannose 6-phosphate-dependent mechanism (half maximal uptake at 0.7 nM) and was "corrective" for abnormal glycosaminoglycan accumulation (half maximal correction at 0.7 pm). The half-life of the recombinant enzyme was 5 days following uptake into Hurler fibroblasts. Production in a 5-liter microcarrier culture system permitted the collection of 15 mg or more per day. Purification to

apparent homogeneity was achieved by sequential chromatography on concanavalin A-Sepharose, heparin-Sepharose, and Sephacryl S-200. Amino acid sequencing of the purified protein proved its identity and purity. The N-terminus contained alanine 26, inconsistent with previous conclusions regarding the site of signal peptide cleavage. Sufficient purified alpha-L-**iduronidase** can now be produced for biochemical studies and for therapeutic attempts in animal models.

L3 ANSWER 21 OF 30 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 9

ACCESSION NUMBER: 1995:30639 BIOSIS
DOCUMENT NUMBER: PREV199598044939
TITLE: Recombinant alpha-L-**iduronidase**: Characterization of the purified enzyme and correction of mucopolysaccharidosis type I fibroblasts.
AUTHOR(S): Unger, Erik G.; Durrant, Jill; Anson, Don S.; Hopwood, John J. (1)
CORPORATE SOURCE: (1) Lysosomal Diseases Res. Unit, Dep. Chemical Pathol., Cenre Med. Genetics, Women's and Children's Hosp., North Adelaide, SA 5006 Australia
SOURCE: Biochemical Journal, (1994) Vol. 304, No. 1, pp. 43-49. ISSN: 0264-6021.
DOCUMENT TYPE: Article
LANGUAGE: English

AB Mucopolysaccharidosis type I (MPS I, Hurler and Scheie syndromes) is an autosomal recessive lysosomal storage disorder that results from a deficiency of the hydrolase alpha-L-**iduronidase** (IDUA) which is involved in the lysosomal degradation of both heparan sulphate (HS) and dermatan sulphate (DS). Patients with MPS I store and excrete large amounts of partially degraded HS and DS. In order to evaluate enzyme replacement therapy for MPS I patients we have expressed human IDUA cDNA in **Chinese Hamster Ovary** (**CHO**)-K1
cells utilizing a plasmid vector that places the cDNA under the transcriptional control of the human polypeptide-chain-elongation factor I-alpha gene promoter. A clonal cell-line that secreted recombinant IDUA in a precursor form at approximately 2.2 mu-g/10-6 cells per day was identified. This enzyme was shown to be endocytosed into cultured MPS I fibroblasts via mannose-6-phosphate receptors and to correct the storage phenotype of these cells by enabling the lysosomal-digestion of accumulated sulphated glycosaminoglycans. The recombinant IDUA had on SDS/PAGE a molecular mass of 85 kDa and was processed to 74 kDa and smaller forms following its uptake by fibroblasts. Milligram quantities of the recombinant IDUA were immunopurified and the enzyme was shown to have pH optimum and kinetic parameters differing from those of the mature enzyme purified from human liver. The specific activity of the recombinant enzyme was shown to increase on dilution and on incubation with reducing agents. This was in contrast to the mature IDUA form (74 kDa) which did not have its activity stimulated by reducing agents or dilution.

L3 ANSWER 22 OF 30 CAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 1993:490104 CAPLUS
DOCUMENT NUMBER: 119:90104
TITLE: Cloning of cDNA for .alpha.-L-**iduronidase** of mammals
INVENTOR(S): Scott, Hamish Steel; Anson, Donald Stewart; Orsborn, Annette Marie; Nelson, Paul Victor; Clements, Peter Roy; Morris, Charles Phillip; Hopwood, John Joseph
PATENT ASSIGNEE(S): Women's and Children's Hospital, Australia
SOURCE: PCT Int. Appl., 46 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9310244	A1	19930527	WO 1992-AU611	19921112
W: AU, CA, JP, KR, US				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, SE				
CA 2099503	AA	19930515	CA 1992-2099503	19911112
AU 9229141	A1	19930615	AU 1992-29141	19921112
AU 649897	B2	19940602		
EP 578790	A2	19940119	EP 1992-923074	19921112
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, SE				
US 6149909	A	20001121	US 1998-191171	19981113
US 6238662	B1	20010529	US 1999-385707	19990830
US 6524835	B1	20030225	US 2000-639696	20000816

PRIORITY APPLN. INFO.:

AU 1991-9490	A	19911114
WO 1992-AU611	A	19921112
WO 1992-AY611	W	19921112
US 1993-84254	B1	19930707
US 1995-494104	B1	19950623
US 1998-191171	B3	19981113

AB A cDNA for mammalian .alpha.-L-**iduronidase** (I) is cloned and the amino acid sequence deduced. A cDNA from human liver encodes seven differential spilicing products: the 65-, 60, and 18-kDa species have a common N-terminus, the 49- and 44-kDa another, and the 74- and 13-kDA yet another. The cDNA may be expressed in a prokaryotic or an eukaryotic cells. Expression in mammalian cells, e.g., **CHO**, gives I with an altered glycosylation pattern, e.g. higher glycosylation than the natural one. Treatment of mucopolysaccharidosis type I using I in various pharmaceutical dosage forms or by gene therapy is claimed. A modified cDNA and the genomic sequence of I were also presented.

L3 ANSWER 23 OF 30 MEDLINE

ACCESSION NUMBER: 94027086 MEDLINE
DOCUMENT NUMBER: 94027086 PubMed ID: 8213840
TITLE: Identification of mutations in the alpha-L-**iduronidase** gene (IDUA) that cause Hurler and Scheie syndromes.
AUTHOR: Scott H S; Litjens T; Nelson P V; Thompson P R; Brooks D A; Hopwood J J; Morris C P
CORPORATE SOURCE: Department of Chemical Pathology, Adelaide Children's Hospital, Australia.
SOURCE: AMERICAN JOURNAL OF HUMAN GENETICS, (1993 Nov) 53 (5) 973-86.
Journal code: 0370475. ISSN: 0002-9297.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199311
ENTRY DATE: Entered STN: 19940117
Last Updated on STN: 19940117
Entered Medline: 19931119

AB Mucopolysaccharidosis type I (MPS-I) is an autosomal recessive genetic disease caused by a deficiency of the lysosomal glycosidase alpha-L-**iduronidase**. Hurler (severe), Scheie (mild), and Hurler/Scheie (intermediate) syndromes are clinical subtypes of MPS-I, but it is difficult to distinguish between these subtypes by biochemical measurements. Mutation analysis was undertaken to provide a molecular explanation for the clinical variation seen in MPS-I. Using chemical cleavage and direct PCR sequencing, we have defined four previously undescribed mutations for MPS-I (delG1702, 1060 + 2t-->c, R89Q, and 678-7g-->a). R89Q and 678-7g-->a were found to be present in 40% of Scheie syndrome alleles. Expression of R89Q demonstrated reduced stability and activity of the mutant protein. The deleterious effect of

R89Q may be potentiated by a polymorphism (A361T) to produce an intermediate phenotype. 678-7g-->a was found to be a mild mutation, since it was present in an index Scheie syndrome patient in combination with a severe allele (W402X). This mutation appears to allow a very small amount of normal mRNA to be produced from the allele which is likely to be responsible for the mild clinical phenotype observed. Both the 5' and 3' splice site mutations (1060 + 2t-->c and 678-7g-->a, respectively) result in high proportions of mature mRNAs containing introns, which has not been observed for other splicing mutations. The frameshift mutation (delG1702) and the 5' splice site mutation (1060 + 2t-->c) are both thought to be associated with severe MPS-I. The identification of these MPS-I mutations begins to document the expected genetic heterogeneity in MPS-I and provides the first molecular explanations for the broad range of clinical phenotypes observed.

L3 ANSWER 24 OF 30 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.

ACCESSION NUMBER: 1993:292176 BIOSIS
DOCUMENT NUMBER: PREV199345010301
TITLE: Overexpression of alpha-L-**iduronidase** for enzyme replacement therapy of Hurler syndrome.
AUTHOR(S): Kakkis, Emil D. (1); Jonas, Adam J. (1); Shull, Robert M.; Neufeld, Elizabeth F.
CORPORATE SOURCE: (1) Harbor-UCLA Med. Center, Dep. Pediatrics, Torrance, CA
SOURCE: Pediatric Research, (1993) Vol. 33, No. 4 PART 2, pp. 129A. Meeting Info.: 103rd Annual Meeting of the American Pediatric Society and 62nd Annual Meeting of the Society for Pediatric Research Washington, D.C., USA May 3-6, 1993 ISSN: 0031-3998.
DOCUMENT TYPE: Conference
LANGUAGE: English

=> d his